

Applicant: Christof NIEHRS et al.
Application No. 09/530,219
Attorney Docket No.2502498-991150 (formerly 40168)

IN THE SPECIFICATION:

Page 1, please replace the fifth full paragraph with the following:

Therefore, the subject matter of the present invention relates to an inhibitor protein of the wnt signal path, the protein comprising at least one of the amino acid consensus sequences I (SEQ ID NO:8) and II (SEQ ID NO:9), indicated in fig. 1.

Please replace the paragraph bridging pages 1 and 2 with the following:

The present invention is based on the applicant's finding that in animals, particularly mammals, very particularly human beings, there is exists a protein which inhibits the wnt signal path. The applicant has found that *in Xenopus laevis* the expression of the wnt gene, Xwnt-8, results in the formation of Siamese twins. This anomaly will be prevented if the above protein is expressed simultaneously. This protein is a secretory protein of about 40 kD. It has at least one of the amino acid consensus sequences I (SEQ ID NO:8) and II (SEQ ID NO:9) rich in cysteine and indicated in fig. 1. Variants of the protein are indicated in the form of their DNAs in figure 2. The applicant has also found that variants of the protein are expressed in differing tissues (cf. Table 1 and figure 3).

Please replace the second full paragraph on page 2 with the following:

In a preferred embodiment, (wnt-I) has the amino acid consensus sequences I (SEQ ID NO:8) and II (SEQ ID NO:9) indicated in fig. 1.

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Please replace the first seven full paragraphs on page 3 with the following:

Fig. 2.1 (DNA from human beings, SEQ ID NO:6) as-phdkk-3 under DSM 11762.

Fig. 2.2 (DNA from chickens, SEQ ID NO:7) is termed pcdkk-3.

Fig. 2.3 (DNA from mice, SEQ ID NO:2) as pmdkk-2 under DSM 11759.

Fig. 2.4 (DNA from human beings, SEQ ID NO:4) as phdkk-2 under DSM 11761.

Fig. 2.5 (DNA from mice, SEQ ID NO:3) as pmdkk-1 under DSM 11758.

Fig. 2.6 (DNA from human beings, SEQ ID NO:5) as phdkk-1 under DSM 11760.

Fig. 2.7 (DNA from *Xenopus laevis*, SEQ ID NO:1) as pRNdkk-1 under DSM 11757.

Please replace the second full paragraph on page 6 with the following:

In particular, the present invention distinguishes itself in that it can be used in tissue-specific fashion. This applies to both diagnosis and treatment. For example, a DNA according to the invention, Dkk-1 (SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5), a corresponding protein and an antibody thereof, respectively, are particularly suitable for tissues, such as brain, heart, vessels, bones, cartilage, connective tissue and eye. Furthermore, a DNA according to the invention, Dkk-2 (SEQ ID NO:2, or SEQ ID NO:4), a corresponding protein and antibody thereof, respectively, are particularly suitable for tissues, kidneys, testes, spleen, ovaries, muscles, uteri, cartilage, eyes and mammas.

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Moreover, a DNA according to the invention, Dkk-3 (SEQ ID NO:6), a corresponding protein and an antibody thereof, respectively, are particularly suitable for tissues, such as brain, heart, vessels, bones, cartilage, eyes, connective tissue, lungs, ovaries, muscles and mammas.

Please replace the paragraph bridging pages 6 and 7 with the following:

Fig. 1 shows the amino acid consensus sequences I (SEQ ID NO:8) and II (SEQ ID NO:9) of a (wnt-I) according to the invention. The indication “-” stands for an amino acid, the number of amino acids being variable when they are provided with an asterisk,

fig. 2 shows the base sequence of seven DNAs coding for (wnt-I) by indicating the bases contributing to the amino acid consensus sequences of (wnt-I),

2.1, SEQ ID NO:6

2.2, SEQ ID NO:7

2.3, SEQ ID NO:2

2.4, SEQ ID NO:4

2.5, SEQ ID NO:3

2.6, SEQ ID NO:5

2.7, SEQ ID NO:1,

fig. 3 shows the expression of three DNAs coding for (wnt-I), Dkk-1 (SEQ ID NO:1), Dkk-2 (SEQ ID NO:2) and Dkk-3 (SEQ ID NO:6), in tissues.

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Please replace the paragraph bridging pages 7 and 8 with the following:

For the preparation of a (wnt-I) according to the invention, the DNA of fig. 2.6 (SEQ ID NO:5), phdkk-1, was provided with Bam HI linkers, then cleaved by Bam HI and inserted in the expression vector pQE-8 (Qiagen) cleaved by Bam HI. The expression plasmid pQ/wnt-I was obtained. Such a plasmid codes for a fusion protein comprising 6 histidine residues (N terminus partner) and a (wnt-I) according to the invention (C terminus partner). pQ/wnt-I was used for transforming E. coli SG 13009 (cf. Gottesman, S. et al., J. Bacteriol. 148, (1981), 265-273). The bacteria were cultured in an LB broth with 100 µg/ml ampicillin and 25 µg/ml kanamycin, and induced with 60 µM isopropyl-β-D-thiogalactopyranoside (IPTG) for 4 h. Lysis of the bacteria was achieved by the addition of 6 M guanidine hydrochloride. thereafter, chromatography (Ni-NTA resin) was carried out with the lysate in the presence of 8 M urea in accordance with the instructions from the manufacturer (Qiagen) of the chromatography material. The bound fusion protein was eluted in a buffer having a pH of 3.5 after its neutralization, the fusion protein was subjected to 18% SDS polyacrylamide gel electrophoresis and dyed with coomassie blue (ccf. Thomas, J.O. and Kornberg, R.D., J. Mol. Biol. 149 (1975), 709-733).